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The state 2 transition in the cyanobacterium *Synechococcus* 6301 can be driven by respiratory electron flow into the plastoquinone pool

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State 1-state 2 transitions in the cyanobacterium *Synechococcus* 6301 were observed using a modulated fluorescence measurement system. State 1 transitions could be reversed even when PS II turnover was completely inhibited by DCMU. In starved cells, in which the rate of respiratory electron flow was greatly decreased, the extent of this reversion to state 2 was reduced. This suggests that state 2 transitions can be driven by respiratory electron flow as well as by PS II turnover and provides further evidence for the intersection of photosynthetic and respiratory electron transport in cyanobacteria. We propose that excitation energy distribution in this organism is controlled by the redox level of plastoquinone or a closely associated component of the electron transport chain.

Photosynthesis *Respiration* *Cyanobacteria* *State 1-state 2 transition* *Electron transport* *Light harvesting*

1. INTRODUCTION

Plants, algae and cyanobacteria are able to adapt rapidly to changes in the spectral quality of actinic light by regulating the distribution of absorbed excitation energy between PS I and PS II so as to maximise the efficiency of light-energy utilisation [1–3]. In green plants the process involves the redistribution of LHC-II between PS I and PS II as a result of protein phosphorylation catalysed by a membrane-bound kinase which is activated when the plastoquinone pool is reduced [4–6]. Since plastoquinone lies between PS I and PS II in the photosynthetic electron transport chain, the redox level of this component provides a sensitive indicator of any imbalance in the turnover of the two photosystems.

The mechanism by which state transitions are controlled in cyanobacteria is controversial, Biggins and co-workers [7] postulate a conformational change arising from localised electrochemical gradients; Satoh and Fork [8] suggest that cyclic electron flow around PS I is the controlling factor. It has recently been shown that the phosphorylation of a number of polypeptides [9], including a phycobilisome component (Sanders, C.E., unpublished), accompanies state transitions in the cyanobacterium *Synechococcus* 6301. The mechanism of state transitions in the cyanobacteria may therefore resemble that in green plants and involve the redistribution of the light-harvesting phycobilisome (the functional equivalent of LHC-II in these organisms) between PS I and PS II in response to phosphorylation [9–11]. The question remains as to how this phosphorylation reaction is controlled,

Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_m fluorescence yield when all PS II centres are closed; light 1, a light exciting predominantly PS I; light 2, a light exciting predominantly PS II; LHC-II, light-harvesting chlorophyll *a/b*-protein complex

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and whether state transitions are controlled in the same way, as would be expected if light-energy distribution were indeed determined by protein phosphorylation.

Here we report results consistent with the control of state transitions in *Synechococcus* 6301 by the redox level of plastoquinone. We suggest that many of the apparent differences in the control of state transitions between cyanobacteria and green plants [3,8] result from the intersection of the photosynthetic and respiratory electron transport chains in cyanobacteria [12–15] and we discuss the implications for the physiological role of state transitions in green algae and cyanobacteria.

2. EXPERIMENTAL

Synechococcus 6301 (Anacystis nidulans) was grown photoautotrophically at 35°C in medium C of Kratz and Myers [16] but with the orthophosphate concentration reduced to 0.44 mM. Cells were subcultured to a concentration of about 1 μg chlorophyll $a \cdot ml^{-1}$ and grown for 4–6 h after subculturing. The cells were starved by aerobic incubation in the dark in a stirred flask at 32°C.

For fluorescence measurements the cell suspension was diluted with medium C where necessary to 2 μg chlorophyll $a \cdot ml^{-1}$; for measurements of oxygen uptake and evolution the cells were harvested by centrifugation (15000 × g, 5 min) and resuspended to give 20 μg chlorophyll $a \cdot ml^{-1}$. Chlorophyll a was determined as in [17] following extraction in 80% acetone. The fraction which was insoluble in 80% acetone was pelleted by centrifugation and resuspended in a small volume of medium C. The absorbance at 630 nm was then used to give an estimate of phycocyanin concentration.

Fluorescence measurements were made in a stirred cuvette at 25°C. Fluorescence was generated using a weak yellow light (light 2) at 1 $W \cdot m^{-2}$ and modulated at 870 Hz. This was provided by an array of yellow LEDs screened by a 650 nm short-pass optical filter (Hansatech, King's Lynn). Fluorescence was detected by a Hansatech photodiode which was screened by a 700 nm interference filter and which was connected to an amplifier locked-in to the frequency of the modulated light 2 [18]. Light 1 at 6 $W \cdot m^{-2}$ was defined by a Corning 5-60 blue filter.

Oxygen uptake and evolution were measured in a Clark-type oxygen electrode (Hansatech) at 25°C. Respiratory oxygen uptake was measured in the dark; photosynthetic oxygen evolution was measured under illumination at about 500 $W \cdot m^{-2}$ with light isolated with a 600 nm long-pass filter. For the Mehler reaction cells were first incubated in the dark at 25°C in the presence of 2 mM methyl viologen for 15–20 min [19]. KCN (1 mM), gramicidin (5 μM) and DCMU (50 μM) were added immediately before the measurement of oxygen uptake. The cells were then illuminated with light isolated by a 600 nm long-pass filter as for the measurement of oxygen evolution, but with the intensity increased to about 1900 $W \cdot m^{-2}$.

3. RESULTS

The fluorescence emission of PS II is dependent in part on absorption cross-section and hence may be used as an indicator of light-state adaptation [20]. Fig.1 shows the effect of starvation on state transitions in *Synechococcus* 6301 cells observed using a modulated fluorescence measurement system as described in [10]. The lock-in amplifier resolves only fluorescence generated by the modulated light 2; only the indirect effects of the non-modulated light 1 are observed. The addition of light 1 always induced a slow rise in fluorescence which we interpret as a state 1 transition. When light 1 was extinguished, there was a rapid rise in fluorescence due to the reduction of the plastoquinone pool and the consequent net closure of PS II reaction centres. This was followed by a slow falling phase most easily interpreted as a state 2 transition. One effect of starvation was to increase the half-time of the state 2 transition (table 1 and fig.1). When starved, dark-adapted cells were illuminated there was an initial rise in fluorescence to a level close to F_m; fluorescence then gradually declined over about 60 min to approximately the initial level seen in unstarved cells. This effect is presumably due to depletion of reductive pentose phosphate pathway intermediates in cells starved in the dark: these intermediates are generated only in the light [21]. In starved cells PS I turnover will therefore be greatly decreased, and the plastoquinone pool will therefore become reduced, leading to a high PS II fluorescence yield which will gradually decline as reductive pentose

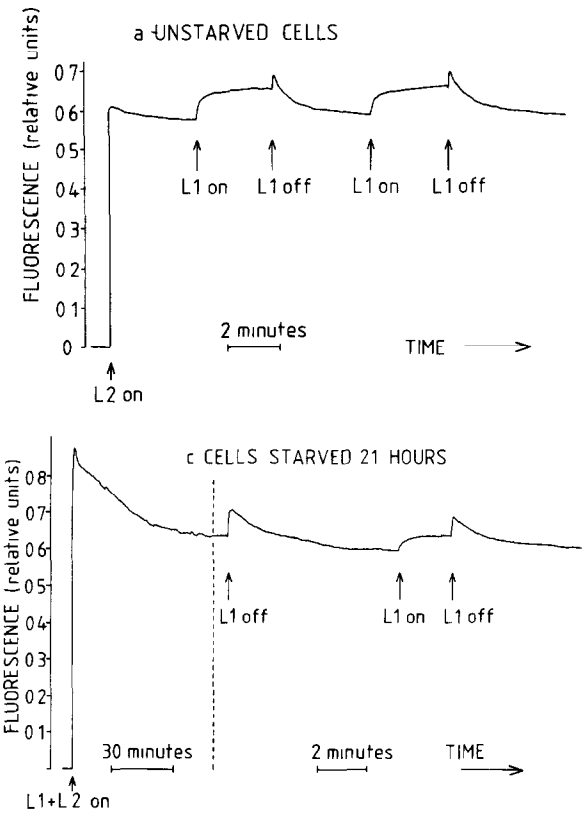

Fig.1. Effect of starvation on state transitions in cells of *Synechococcus* 6301. (a) Photoautotrophically grown cells. (b) Cells starved for 21 h. Conditions as described in section 2. The fluorescence scale is relative to F_m in state 1.

phosphate pathway intermediates are generated in response to light. The rate of PS I turnover then increases and the plastoquinone pool becomes more oxidised. In starved cells, there was a corresponding lag in oxygen evolution following illumination before the maximum rate was attained (not shown).

Fig.2 shows the effect of adding DCMU to unstarved cells (fig.2a) and to starved cells (fig.2b,c) in state 2. DCMU induces a fluorescence rise to F_m since it prevents photochemical quenching of PS II fluorescence by blocking electron transport from PS II to plastoquinone. In starved cells (fig.2c), the addition of DCMU causes a rise to F_m followed by a slower rising phase apparently due to a state 1 transition. The subsequent addition of light 1 causes only a small increase in fluorescence yield. In unstarved cells, however, the addition of DCMU results in only a small slow-phase rise which is subsequently reversed (fig.2a). The addition of light 1 then produces a pronounced slow rise in fluorescence, which is reversed when the light is extinguished. Since the

Table 1

Effect of starvation on the half-times (estimated from semi-log plots) of the state transitions shown in figs 1 and 2

Starvation time (h)	Half-time (s)			
	State 1 (− DCMU)	State 2 (− DCMU)	State 1 (+ DCMU)	State 2 (+ DCMU)
0	14	30	14	25
2	10	30	14	25
21	14	50	–	–

After 21 h starvation state transitions in the presence of DCMU were too small for their half-times accurately to be assessed

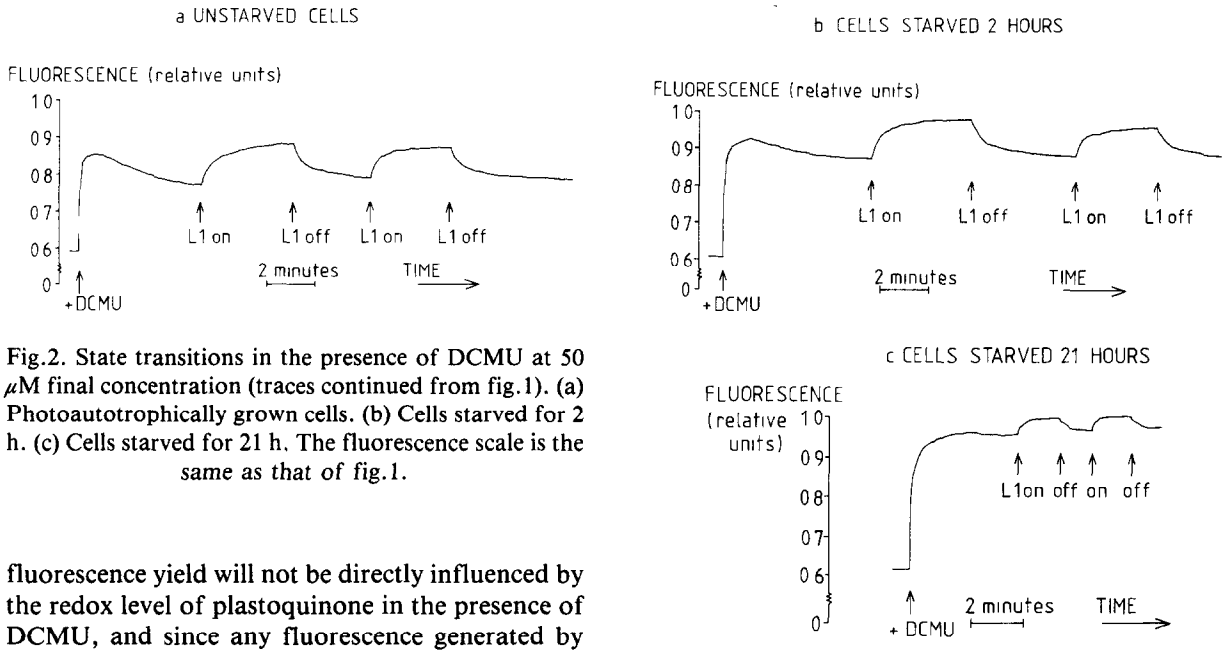

Fig.2. State transitions in the presence of DCMU at 50 μM final concentration (traces continued from fig.1). (a) Photoautotrophically grown cells. (b) Cells starved for 2 h. (c) Cells starved for 21 h. The fluorescence scale is the same as that of fig.1.

fluorescence yield will not be directly influenced by the redox level of plastoquinone in the presence of DCMU, and since any fluorescence generated by light 1 is not detected this effect can only be interpreted as a state 1 transition.

Table 2 shows the effect of starvation on the rates of dark oxygen uptake and of the Mehler reaction, in which methyl viologen acts as a PS I electron acceptor. The reduced methyl viologen then reduces oxygen to hydrogen peroxide, resulting in net oxygen uptake which is increased in the presence of a catalase inhibitor [19,22]. Starvation results in a pronounced decrease both in the rate of dark oxygen uptake and in the rate of the Mehler reaction in the presence of DCMU and in the absence of an added electron donor. Since DCMU at 50 μM was sufficient completely to suppress oxygen evolution, the reducing equivalents necessary for the reduction of methyl viologen must under these conditions be supplied by respiratory electron flow into the plastoquinone pool. KCN at 1 mM serves to inhibit endogenous catalase activity and Calvin cycle turnover.

Table 2

Effect of starvation on the rate of respiratory electron flow in cells of *Synechococcus* 6301

Starvation time (h)	Respiration rate	Mehler reaction (+ DCMU)
0	7.6	18.0
2	5.7	15.5
21	1.0	9.0

Conditions as described in section 2. Rates given in μmol $O_2 \cdot (\text{mg chlorophyll})^{-1} \cdot h^{-1}$

Starvation did not measurably change the levels of either chlorophyll *a* or phycocyanin (not shown).

4. DISCUSSION

The present results suggest that the state 2 transition in the cyanobacterium *Synechococcus* 6301 can be driven by respiratory electron flow as well as by light. We have used the Mehler reaction in the presence of DCMU and KCN as an indicator of the potential rate of respiratory electron flow into the plastoquinone pool. In unstarved cells, the plastoquinone pool is largely reduced in the dark [3,13] suggesting that, under these conditions, the rate-limiting step in dark respiratory electron transport lies after plastoquinone. The rate of dark oxygen uptake is therefore unlikely to be a reliable measure of the potential rate of respiratory electron flow into platoquinone. We found that starvation substantially reduced both the rate of respiratory oxygen uptake and the rate of the Mehler reaction in the presence of DCMU (table 2).

The effect of starvation on state transitions in the presence of DCMU is consistent with the control of state transitions in this organism by the redox level of plastoquinone. In starved cells, the rate of respiratory electron flow into plastoquinone is decreased (table 2). Following the addition of DCMU the weak modulated light 2 will then drive PS I turnover sufficiently fast to oxidise the plastoquinone pool and hence induce a state 1 transition, seen as the slow phase of the fluorescence rise (fig.2c). Since the plastoquinone pool is already largely oxidised, the addition of light 1 has little further effect. In unstarved cells (fig.2a), however, the rate of respiratory electron flow into the plastoquinone pool is sufficient to keep plastoquinone largely reduced even in the presence of DCMU. Hence the addition of DCMU does not induce a significant state 1 transition. The subsequent addition of light 1 drives PS I turnover faster than the rate of respiratory electron flow, thus oxidising the plastoquinone pool and thereby causing a state 1 transition, seen as a fluorescence rise (fig.2a). This is reversed when light 1 is extinguished, since respiratory electron flow reduces the plastoquinone pool again, inducing a state 2 transition. The maximum fluorescence yield seen in the presence of DCMU and light 1 is increased by 2 h starvation (fig.2b); 21 h starvation has little further effect (fig.2c). This suggests that in unstarved cells respiratory electron flow is too rapid to allow our light 1 to induce a complete state 1 transition in the presence of DCMU: this is seen only in starved cells. Prolonged (21 h) starvation inreases the half-time of the state 2 transition seen in the absence of DCMU (table 1), presumably because electron flow into the plastoquinone pool is then slow enough to be rate limiting for the state 2 transition. This result implies that our weak, modulated light 2 is ineffective in driving state 2 transitions; the state 2 transitions which we observe are due principally to respiratory electron flow. Aoki and Katoh [13] have reported that light 2 is rather ineffective in reducing the plastoquinone pool in cyanobacteria presumably either because of a preponderance of PS I over PS II reaction centres [23] or else because of a high degree of spillover of excitation energy from PS II to PS I in a laterally homogeneous thylakoid membrane. Starvation did not significantly affect the half-time of the state 1 transition (table 1).

Our results are difficult to reconcile with the suggestions that state transitions in cyanobacteria are controlled by cyclic electron flow around PS I [8] or by localised electrochemical gradients [7]. Starvation does affect the initial rate of non-cyclic electron flow by lowering the levels of PS I electron acceptors. However, following a period of light adaptation, normal levels of PS I turnover are restored, as indicated by the steady-state fluorescence yield (fig.1b). The dark state in cyanobacteria has been observed to be state 2 [3,10], in contrast to higher plants [24]. This is consistent with the control of state transitions by the redox state of plastoquinone, since respiratory electron transport in cyanobacteria will keep the plastoquinone pool reduced in the dark [13]. There is evidence for a respiratory electron transport chain, intersecting with the photosynthetic electron transport chain at the level of plastoquinone, in the chloroplasts of some green algae [25–27]. This may be the explanation for the dark state 2 which has been observed under some conditions in certain of these organisms [28,29].

We propose that state transitions in the cyanobacteria result from the phosphorylation of light-harvesting polypeptides [9,30] by a protein kinase whose activity is controlled by the redox state of plastoquinone, or of another electron carrier which is common to the photosynthetic and respiratory electron transport pathways and which lies between the two photosystems. Scherer and Böger [31] have found evidence for a flow of reducing equivalents from oxidisable substrates into the respiratory electron transport chain even in the light, suggesting that reducing equivalents generated by the oxidative pentose phosphate pathway are cycled through PS I in the light. Our model implies that state transitions in the cyanobacteria play a part in regulating the interaction of respiratory and photosynthetic metabolism, as well as in balancing the distribution of excitation energy between the two photosystems. We conclude that control of state transitions by the redox level of plastoquinone allows the distribution of absorbed excitation energy between the two photosystems to be adjusted to match the cell's metabolic demands because the redox level of plastoquinone is influenced by respiratory as well as by photosynthetic electron flow.

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